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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Daniel E. H. AFAR, *et al.*

Serial No.: 09/455,486

Filing Date: 6 December 1999

For: NOVEL SERPENTINE TRANSMEMBRANE ANTIGENS EXPRESSED IN HUMAN CANCERS AND USES THEREOF

Examiner: Gary B. Nickol, Ph. D.

Group Art Unit: 1642

DECLARATION OF PIA M. CHALLITA-EID
UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Pia M. Challita-Eid, declare as follows:

1. I have a Ph.D. in Microbiology from University of Southern California, did post doctoral work at University of California at Los Angeles, and was a faculty member at the University of Rochester. I have been practicing in the field of molecular biology for over 10 years. At Agensys, I am the Group Leader of Gene Discovery. In my position at Agensys, I have responsibility for evaluating the levels of expression of various genes in tissues. A copy of my *curriculum vitae* is enclosed as Exhibit 1.

2. Although I did not myself conduct them, I have direct personal familiarity with the conduct of the experiments described below.

3. In accordance with standard procedures recognized as significant by workers engaged in assessing expression levels of proteins associated with tissues, in particular, malignant tissues, expression of STEAP-2 was assessed in normal and patient cancer tissues using semi-quantitative PCR.

4. First, normal tissues and pools of tissue specimens from cancer patients were tested. First strand cDNA was generated from normal stomach, normal brain, normal heart, normal liver, normal skeletal muscle, normal testis, normal prostate, normal bladder, normal kidney, normal colon, normal lung, normal pancreas, and a pool of cancer specimens from prostate cancer patients, bladder cancer patients, kidney cancer patients, colon cancer patients, lung cancer patients, pancreas cancer patients, and a pool of two patient prostate metastases to the lymph node. Normalization was performed by PCR using primers to actin. Semi-quantitative PCR, using primers directed to STEAP-2, was performed at 26 and 30 cycles of amplification. Samples were run on an agarose gel, and PCR products were quantitated using the AlphaImager™ software. Results show strong expression of STEAP-2 in normal prostate and in prostate cancer. Increased expression was also detected in bladder cancer, kidney cancer, colon cancer, lung cancer, pancreas cancer, breast cancer and cancer metastases as well as in the prostate cancer metastases to lymph node specimens, compared to all normal tissues tested, as seen in Exhibit 2.

5. Panels containing individual cancer patient specimens were also tested. First strand cDNA was prepared from a panel of patient cancer specimens representing lung, ovary, prostate, bladder, cervix, uterus and pancreas. Normalization was performed by PCR using primers to actin. Semi-quantitative PCR, using primers to STEAP-2, was performed at 26 and 30 cycles of amplification. Samples were run on an agarose gel, and PCR products were quantitated using the AlphaImager™ software. Expression was recorded as absent, low, medium or strong. Results show expression of STEAP-2 in the majority of all patient cancer specimens tested as seen in Exhibit 3.

6. Stomach cancer patient specimens were also tested. RNA was extracted from normal stomach (N) and from 10 different stomach cancer patient specimens (T) as shown in panel A of Exhibit 4. Northern blot with 10 μg of total RNA/lane was probed with STEAP-2 sequence. Results show strong expression of STEAP-2 in the stomach tumor tissues and lower expression in normal stomach. The lower panel represents ethidium bromide staining of the blot showing quality of the RNA samples. Panel B (of Exhibit 4) shows expression of STEAP-2 in a panel of human stomach cancers (T) and their respective matched normal tissues (N) on RNA dot blots. STEAP-2 was detected in seven out of eight stomach tumors but not in the matched normal tissue.

7. To demonstrate that antibodies can be raised against STEAP-2 that detect expressed protein, 293T cells were transfected with a vector wherein STEAP-2-encoding DNA optimized according to Mirzabekov, *et al.* (1999) was fused to GFP and cloned into pcDNA 3.1 (Invitrogen, Carlsbad, CA). The expressed fusion protein is STEAP-2 with green fluorescent protein (GFP) fused to the carboxy terminus. The vector also contains bovine growth hormone polyadenylation signal and transcription termination sequence, and expression is driven by the cytomegalovirus (CMV) promoter. The vector also contains an SV40 origin for episomal replication, a ColE1 origin of replication, and genes for neomycin resistance and ampicillin resistance. A comparable STEAP1.GFP vector was used as a positive control, and as a negative control an empty vector was used. Forty hours later, cell lysates were collected. Samples were run on an SDS-PAGE acrylamide gel, blotted and stained with either anti-GFP antibody, anti-STEAP-2 antibody generated against amino acids 198-389, or anti-STEAP-2 antibody generated against amino acids 153-165. The blot was developed using the ECL™ chemiluminescence kit and visualized by autoradiography. Results show expression of the expected STEAP-2.GFP fusion protein as detected by the anti-GFP antibody and by antibodies raised against STEAP-2 as shown in Exhibit 5. (In Exhibit 5, clone A12 and clone B12 each contain the STEAP-2/GFP vector described above.)

8. Further evidence that STEAP-2 protein is expressed is shown in Exhibit 6, panels A and B, by detection of the GFP fusion using flow cytometry (panel A) or fluorescence microscopy (panel B). Panel A shows flow cytometric peaks attributable to the expression of the protein and panel B shows production of detectable protein in transfected cells. The protein appears localized to the perinuclear area and to the cell membrane.

9. Confirmation that STEAP-2 protein is expressed in cancer tissues is shown in Exhibit 7. STEAP-2 protein was detected by immunohistochemistry in STEAP-2.pcDNA3.1 transfected 293T cells (Panel A) and in patient prostate and lung cancer specimens (Panel B). In Panel A, STEAP-2.pcDNA3.1 vector was transfected into 293T cells. The cells were stained with the anti-STEAP2 antibody described in Exhibit 5. Results showed specific staining of STEAP-2 protein in the STEAP-2 transfected 293T cells (A1), but not in the parental 293T cells (A2) which do not express this protein. In Panel B, prostate cancer (B1) and lung cancer (B2) patient specimens were stained with the anti-STEAP-2 antibody. Results showed expression of the STEAP-2 protein in prostate and lung cancer patient specimens.

10. In light of the results above, I conclude as follows. STEAP-2 protein is expressed in cells. Further, STEAP-2 is a useful marker for cancer tissues; STEAP-2 was detected in patient cancer specimens by immunoreactivity with antibodies raised against STEAP-2 epitopes.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Executed at Santa Monica, California, on 6 February 2004.

Pia M. Challita-Eid

Curriculum Vitae

PIA M. CHALLITA-EID, PH.D

Personal information

Home Address:	4229 Colbath Avenue Sherman Oaks, CA 91423
Phone:	(626) 233-9434
Work Address:	University of Rochester Cancer Center 601 Elmwood Avenue, Box #704 Rochester, NY 14642
Email address:	*eid@frontiernet.net*
Date of Birth:	June 17, 1966
Marital status:	Married to Emile R. Eid

Education:

B.S. Biology	American University of Beirut-Lebanon 1984-1987
M.S. Microbiology	American University of Beirut-Lebanon 1987-1989
Ph.D. Microbiology	University of Southern California Department of Microbiology January 1990 - June 1994
Sponsor:	Donald B. Kohn, M.D., Associate, Professor Departments of Pediatrics and Microbiology Division of Research Immunology and Bone Marrow Transplantation Childrens Hospital of Los Angeles University of Southern California, California USA
Postdoctoral fellowship	University of California Los Angeles Department of Hematology-Oncology September 1994 - December 1995
Sponsor:	Joseph D. Rosenblatt, M.D., Assistant Professor School of Medicine Department of Hematology-Oncology University of California, Los Angeles, California

Exhibit 1

Appointments:

Senior Instructor	University of Rochester Cancer Center Department of Oncology January 1996- June 1998
Assistant Professor in Medicine, Microbiology & Immunology	University of Rochester Cancer Center Hematology/Oncology Unit July 1998- Present

Languages:

Fluent in English, French and Arabic.

Students and Technicians Mentored:

1. Skelton Diane, Research Associate, 1992-1994.
2. El-Khoueiry Anthony, Undergraduate student, Summer 1992 and 1993. Currently resident in internal medicine at USC.
3. Poles Tina, Research Associate, 1996-1998.
4. Mosammaparast Nima, Undergraduate student, June 1996 - September 1997. Currently enrolled in Medical School.
5. Zoric Bojan, Undergraduate student, June 1997-June 1998. Currently enrolled in Medical School.
6. Rimel BJ, Research Associate, June 1998-June 1999.
7. Vicki Houseknecht, Research Associate, June 1999 – present.
8. Facciponte John, Graduate student in the Microbiology and Immunology Department at the University of Rochester, January 1998 - present.
9. Kyung Yi, Graduate Student in Microbiology, January 1999 - present.
10. Anagha Joshi, Post-doctoral fellow, October 1999 - present.

Patents:

1) "Retroviral Vectors for Expression in Embryonic Cells", reference number 08/361,112 filed December 1994.
2) "Chimeric Proteins for the Stimulation of a Tumor-Specific Immune Response", application in progress.

Invited Presentations:

October 1994 "Retroviral Vector Expression in Murine Stem Cells". Department of Hematology-Oncology, UCLA Gene Therapy Program, Los Angeles, California.

October 1997 "Antibody Fusion Proteins for the Specific Recruitment and Activation of an Anti-Tumor immune Response". Childrens Hospital of Los Angeles, Los Angeles, California.

February 1998 Regional Cancer Center Consortium for Biological Therapy. Roswell Park Cancer Institute, Buffalo, New York.

July 1998 American Cyanamid Company. Lederle-Praxis Biologicals Division, Rochester, New York.

October 1999 "Monoclonal Antibody Technology in the Era of Genetic Engineering" Brazilian Meeting on Biosafety and Transgenic Products, Rio De Janeiro, Brazil.

June 1999 "Breast Cancer Research in the Era of Genetic Engineering", Breast Cancer Coalition of Rochester, Rochester, NY.

Awards:

Graduate Student Research Forum Award. Silencing of retroviral vectors after transduction of hematopoietic stem cells is associated with methylation. Graduate Student Research Forum Poster Session. USC Medical School, Los Angeles, California, 1993.

Presidential Award. Society of Biological Therapy, Pasadena, California, October 1997.

Merit Award. American Society of Clinical Oncology, California, May 1998.

Grants/Funds:

1) Jonsson Cancer Center Foundation/UCLA
Fellowship Seed Grant
Title: "Antigen Processing in Human Neural Crest Tumors"
Effective Dates: 11/1/95-10/31/96

Amount: $27,707

2) Rochester Area Foundation
Lucille B. Kesel Fund for the Advancenent of Cancer Research
Title: "Antibody Fusion Proteins for Eradication of Minimal Residual Disease"
Effective Dates: 1/1/98-12/31/98
Amount: $8,000

3) University of Rochester Cancer Center
Interim and Pilot Project Funding
P.I.: Joseph D. Rosenblatt, M.D.
Co-P.I.: Pia M. Challita-Eid, Ph.D.
Title: "Antibody Fusion Proteins for the Therapy of Cancer".
Effective Dates: 1/1/98-12/31/98
Amount: $25,000

4) Sinsheimer Scholar Award
Title: "Genetically-Engineered Chemokine Antibody Fusion Proteins for Breast and Ovarian Cancer Therapy"
Effective Dates: 7/1/98-6/30/01
Amount: $40,000/year

5) NIH/NCI
P.I.: Joseph D. Rosenblatt, M.D.
Co-P.I.: Pia M. Challita-Eid, Ph.D.
Title: "Recruitment and Activation of an Anti-tumor Response using Antibody-Fusion Proteins"
Effective Dates: 12/1/98-11/30/03
Amount: $191,046/year

6) NIH/NCI - Rapid Access to Intervention Development (RAID)
Title: "Preclinical Development of a B7.1 Anti-HER2/neu Antibody Fusion Protein"
Effective Date: Approved April, 1999
Amount: Not applicable

7) ACS Institutional grant
Title: "Chemokine Directed Targeting of Cytotoxic TALL-104 Cells"
Effective Dates: 9/1/99-8/30/00
Amount: $8,000

8) Breast Cancer Coalition of Rochester
Title: "Breast Cancer Research"
Date: 9/99
Amount: $1,000

Publications:

Gersuk GM, Westermark B, Mohabeer AJ, **Challita PM**, Pattamakom S, and Pattengale, PK. Inhibition of human natural killer cell activity by platelet- derived growth factor (PDGF). III. Membrane binding studies and differential biological effects of recombinant PDGF isoforms. *Scand J Immunol* 33: 521-532, 1991.

Gersuk GM, Carmel R, **Challita PM**, Rabinowitz AP, and Pattengale PK. Quantitative and functional studies of impaired natural killer (NK) cells in patients with myelofibrosis, essential thrombocytopenis, and polycythemia vera. I. A potential role for platelet-derived growth factor in defective NK cytotoxicity. *Nat Immun* 12: 136-151, 1993.

Challita PM, and Kohn DB. Lack of expression from a retroviral vector in murine hematopoietic stem cells is associated with methylation *in vivo*. *Proc Natl Acad Sci (USA)* 91: 2567-2571, 1994.

Krall W, **Challita PM**, Perlmutter L, Skelton D, and Kohn DB. Cells expressing human glucocerebrosidase from a retroviral vector repopulate macrophages and central nervous system microglia after murine bone marrow transplantation. *Blood* 83: 2737-2748, 1994.

Challita PM, Skelton D, Yu XJ, El-Khoueiry A, Yu X-J, Weinberg KI, and Kohn DB. Multiple modifications in *cis* elements of the long terminal repeat of retroviral vectors leads to increased expression and decreased DNA methylation in embryonic carcinoma cells. *J Virol* 69: 748, 1995.

Ucar K, Seeger RC, **Challita PM**,Watanabe CT, Yen TL, Morgan JP, Amado R, Chou E, McCallister T, Barber JR, Jolly DJ, Reynolds P, Gangavalli R, and Rosenblatt JD. Sustained cytokine production and immunophenotypic changes in human neuroblastoma cell lines transduced with a human gamma interferon vector. *Cancer Gene Therapy* 2: 171, 1995.

Lu Y, Planelles V, Palaniappan C, Li X, **Challita-Eid PM**, Amado R, Stephens D, Kohn DB, Bakker A, Day B, Bambara RA, and Rosenblatt JD. Inhibition of HIV-1 replication using a mutated tRNALys3 primer. *J Biol Chem* 272: 14523, 1997.

Challita-Eid PM, Penichet ML, Shin SU, Poles T, Mosammaparast N, Mahmood K, Slamon DJ, Morrison SL, and Rosenblatt JD. A B7.1-antibody fusion protein retains antibody specificity and ability to activate*via* the T cell costimulatory pathway. *J Immunol* 160: 3419-3426, 1998.

Challita-Eid PM, Abboud CN, Morrison SL, Penichet ML, Rosell KE, Poles T, Hilchey SP, Planelles V, and Rosenblatt JD. A RANTES- antibody fusion protein retains antigen specificity and chemokine function. *J Immunology* 161: 3729, 1998.

Challita-Eid PM, Rosenblatt JD, Day B, Rimel BJ and Planelles V. Inhibition of HIV-1 infection with a RANTES.IgG3 fusion protein. *AIDS Research and Human Retroviruses* 14:1617, 1998.

Mahmood K, Federoff HJ, **Challita-Eid PM**, Day B, Haltman M, Atkinson M, Planelles V, and Rosenblatt JD. Eradication of pre-established lymphoma using HSV amplicon vectors. *Blood* 93: 643, 1999

Penichet ML, **Challita-Eid PM**, Shin S-U, Sampogna S, Rosenblatt JD, and Morrison SL. Establishment of human HER2/neu expressing tumors. *Laboratory Animal Science* 49: 179-88, 1999.

Penichet ML, Dela Cruz JS, **Challita-Eid PM**, Rosenblatt JD, and Morrison SL. A Murine B cell lymphoma expressing human HER2/neu undergoes spontaneous tumor regression and elicits anti-tumor immunity. *Manuscript submitted.*

Hilchey SP, Rosebrough SF, Morrison SL, Rosenblatt JD, and **Challita-Eid PM**. Specific targeting and stimulation of in vivo anti-tumor response using a B7.1 T-cell costimulatory antibody fusion protein. *Manuscript in preparation.*

Selected Abstracts and Presentations:

Challita PM, El-Khoueiry AB, and Kohn DB. Silencing of retroviral vectors after transduction of murine hematopoietic stem cells is associated with methylation. *Blood* 80 (10 Suppl. 1): 168a, 1992.

Challita PM, Cook C, Sender LS, and Kohn DB. Novel retroviral vectors for consistent expression after transduction into hematopoietic stem cells. Keystone Symposium on Gene Therapy, Keystone, Colorado, 1993.

Challita PM. Retroviral vector expression in murine stem cells. Presentation. Division of Hematology-Oncology, University of California Los Angeles, October, 1994.

Challita PM, Shin S-U, Penichet M, Mahmood K, Poles TM, Rosell KE, Abboud CN, Morrison SL, Rosenblatt JD. Novel Antibody Fusion Proteins for the Stimulation of a Tumor-Specific Immune Response. Keystone Symposium on Cellular Immunology and Immunotherapy of Cancer, Copper Mountain, Colorado, January 1997.

Penichet ML, **Challita PM**, Shin S-U, Slamon DJ, Rosenblatt JD, and Morrison SL. In vivo properties of two human her2/neu expressing murine cell lines in immunocompetent mice. Mutlidisciplinary Approaches to Cancer Immunotherapy, Bethesda, Maryland, June 1997.

Challita PM, Abboud CN, Rosell KE, Penichet ML, Poles T, Mahmood K, Morrison SL, and Rosenblatt JD. Characterization of a RANTES-antibody fusion protein for cancer immunotherapy. Mutlidisciplinary Approaches to Cancer Immunotherapy, Bethesda, Maryland, June 1997.

Horwitz S, Rosenblatt JD, Mosammaparast N, Poles T, Abboud CN, and **Challita PM**. Gene-modified EL4 cells expressing the chemokine RANTES protects from tumor growth and stimulates an anti-tumor cytotoxic T-lymphocyte response *in vivo*. Mutlidisciplinary Approaches to Cancer Immunotherapy, Bethesda, Maryland, June 1997.

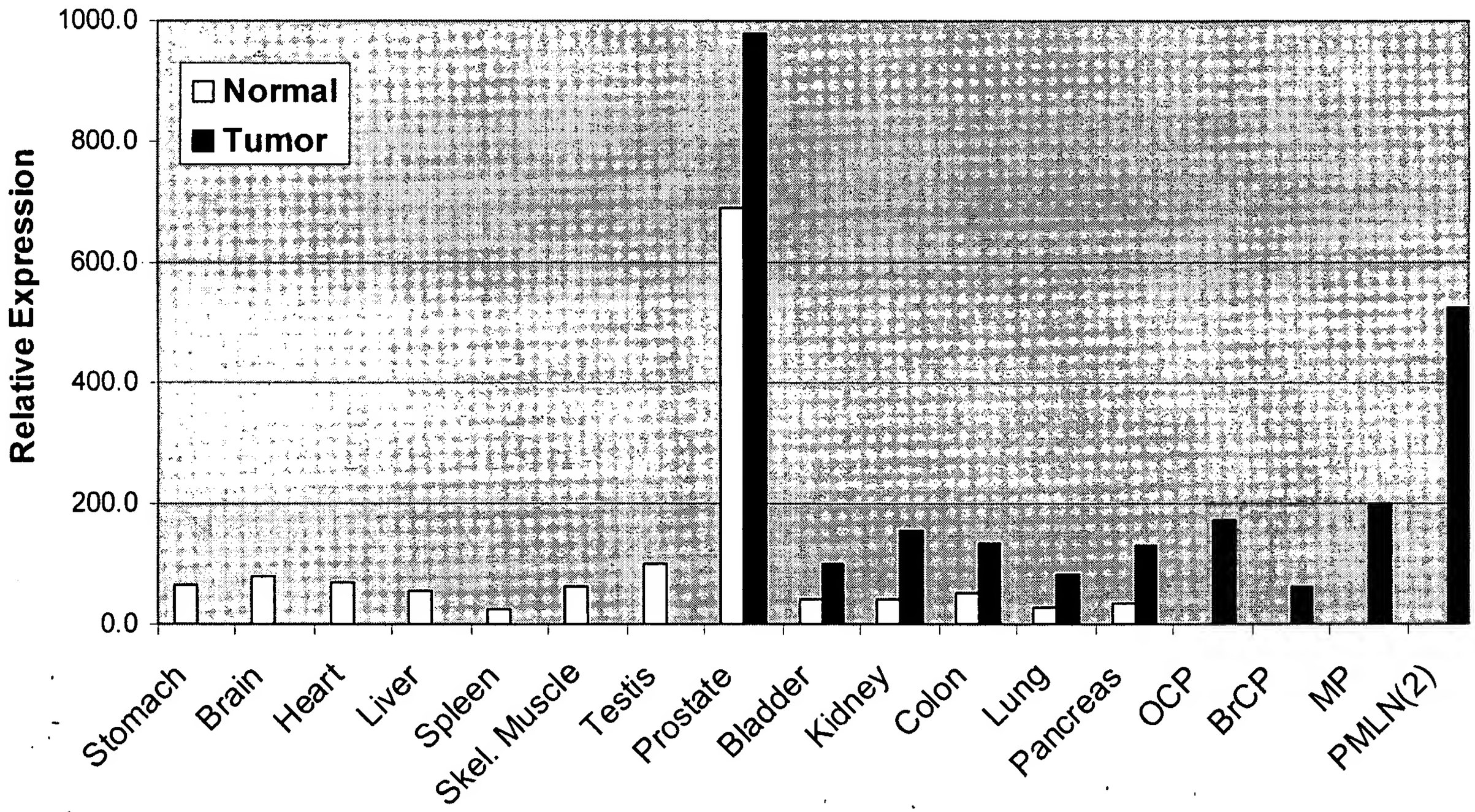
Challita-Eid PM, Morrison SL, Penichet ML, Rosenblatt JD. Antibody-T cell costimulatory ligand fusion protein for the stimulation of a specific anti-tumor immune response. American Society of Hematology, San Diego, California, December 1997.

Challita-Eid PM, Abboud CN, Penichet ML, Rosell KE, Morrison SL, Rosenblatt JD. Antibody fusion proteins for the recruitment and activation of an anti-tumor immune response. American Association for Cancer Research. New Orleans, Louisiana, March 1998.

Challita-Eid PM, Hilchey Shannon P., and Rosenblatt Joseph D. An anti-HER2/neu RANTES fusion protein induces effector cell infiltration to the site of HER2/neu expressing tumors. AACR/NCI/EORTC Molecular Targets and Cancer Therapeutics, Washington DC, November 1999.

Facciponte JG, Rosenblatt JD, H.J.Federoff HJ, **Challita-Eid PM**. Herpes simplex virus (HSV) amplicon-mediated gene transfer of tumor associated antigens into bone marrow derived dendritic cells. Keystone Symposium on Cellular Immunity and Immunotherapy of Cancer, Santa Fe, New Mexico, January 2000.

Exhibit 2:



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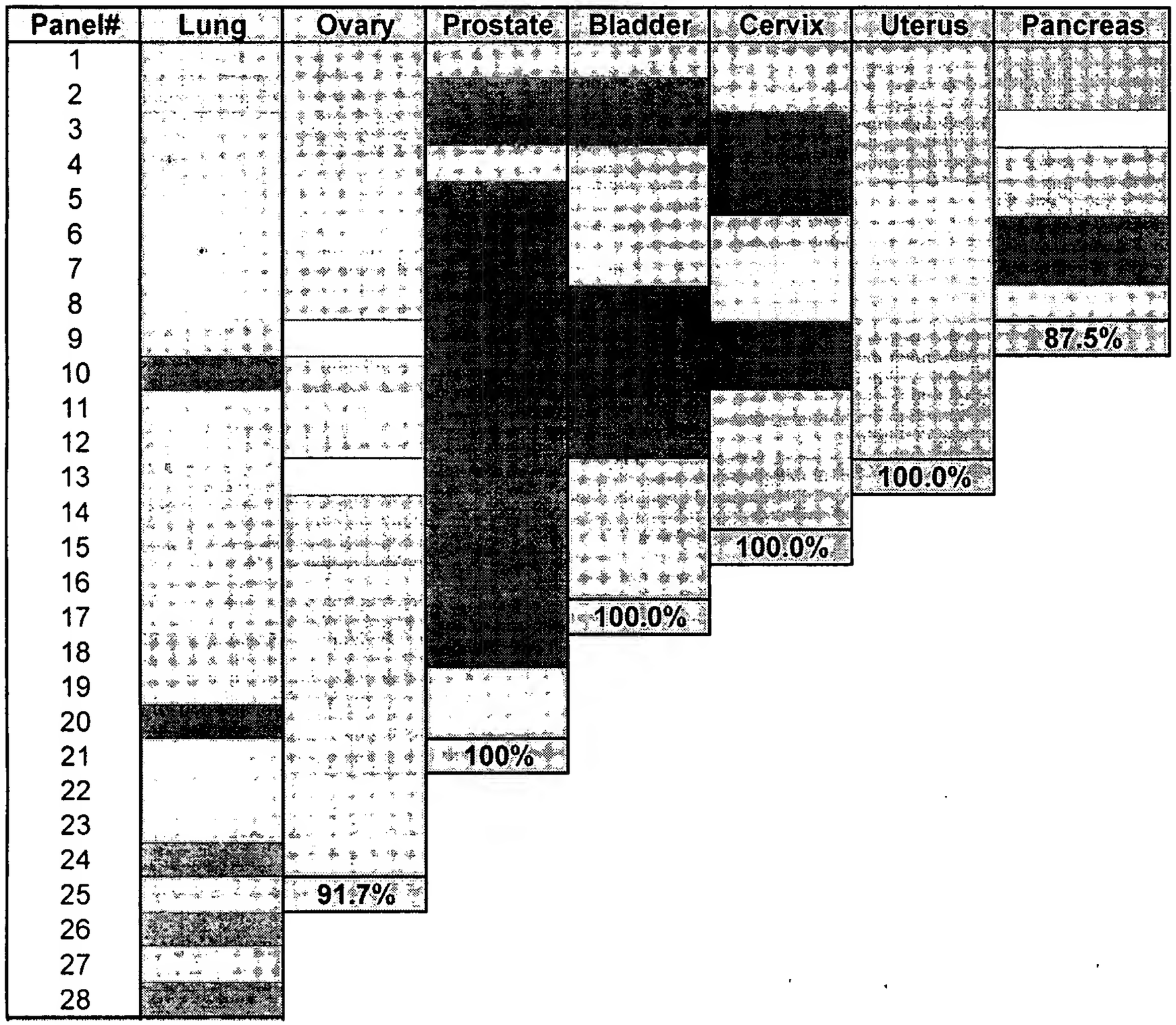
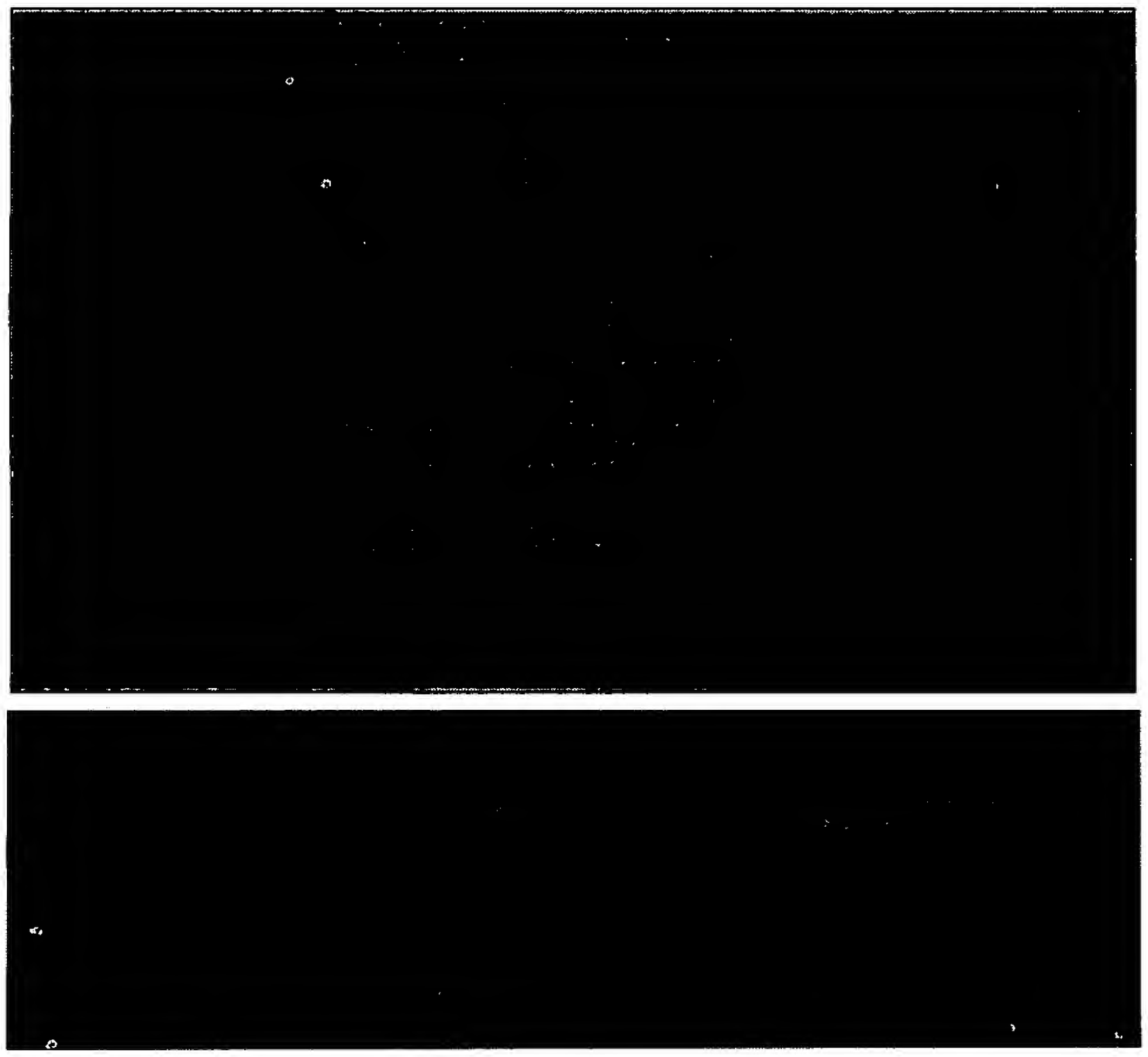
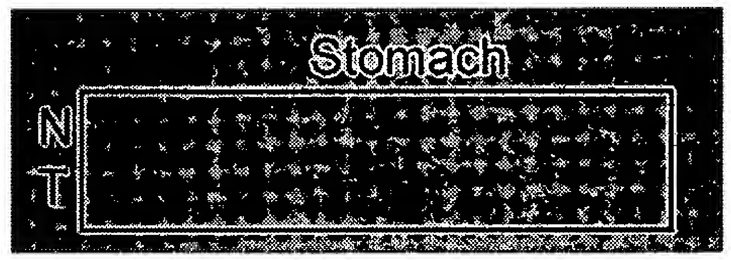


Exhibit 4:

A

N T1 T2 T3 T4 T5 T6 T7 T8 T9 T10

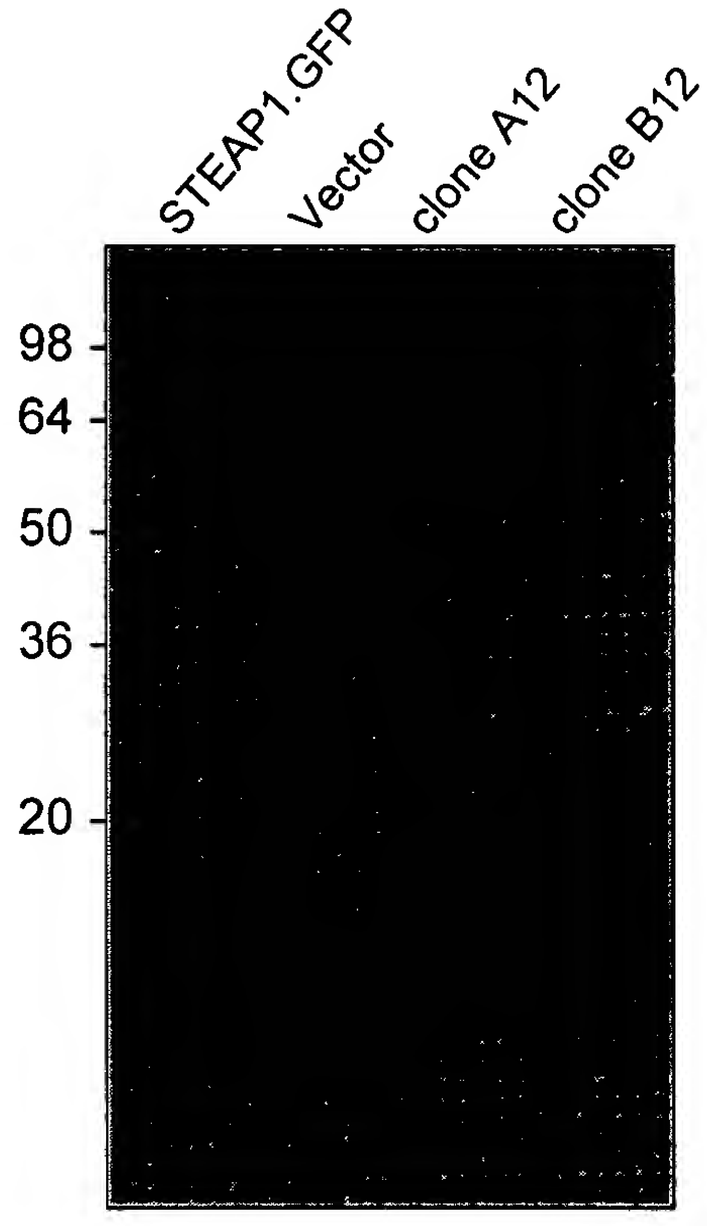




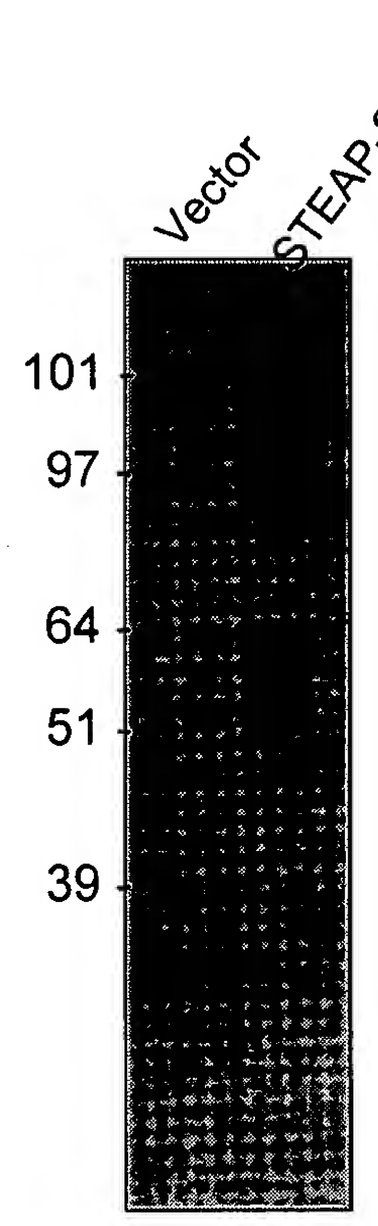
B

Exhibit 5:

A. Anti-GFP



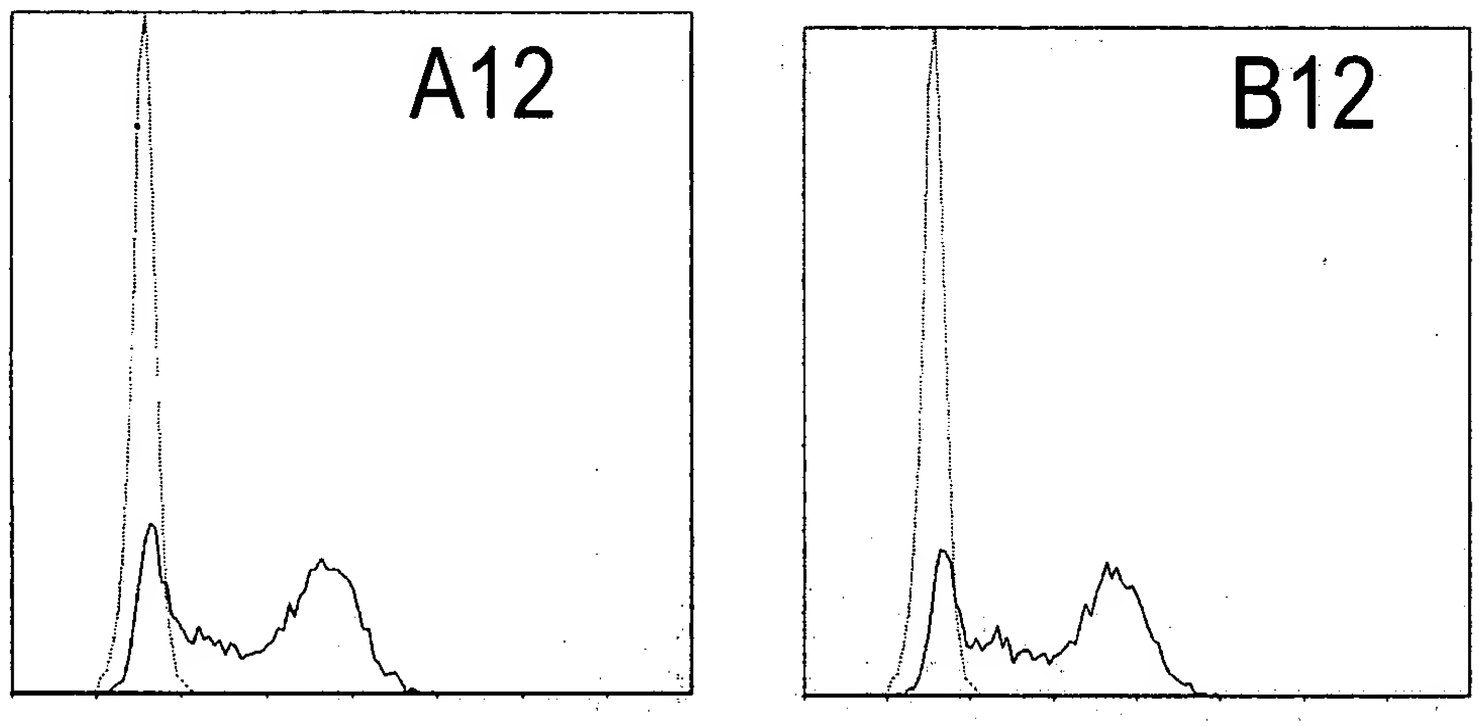
B. pAb (GST aa193-389)



C. pAb (peptide aa15

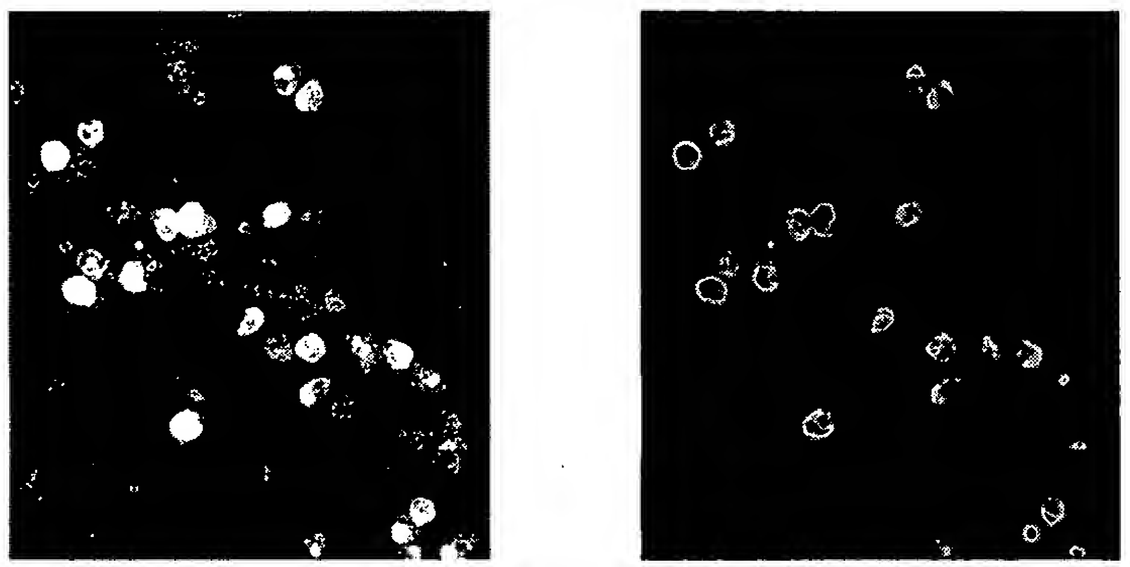
Exhibit 6: Expression of 98P4B6.EGFP Fusion Protein

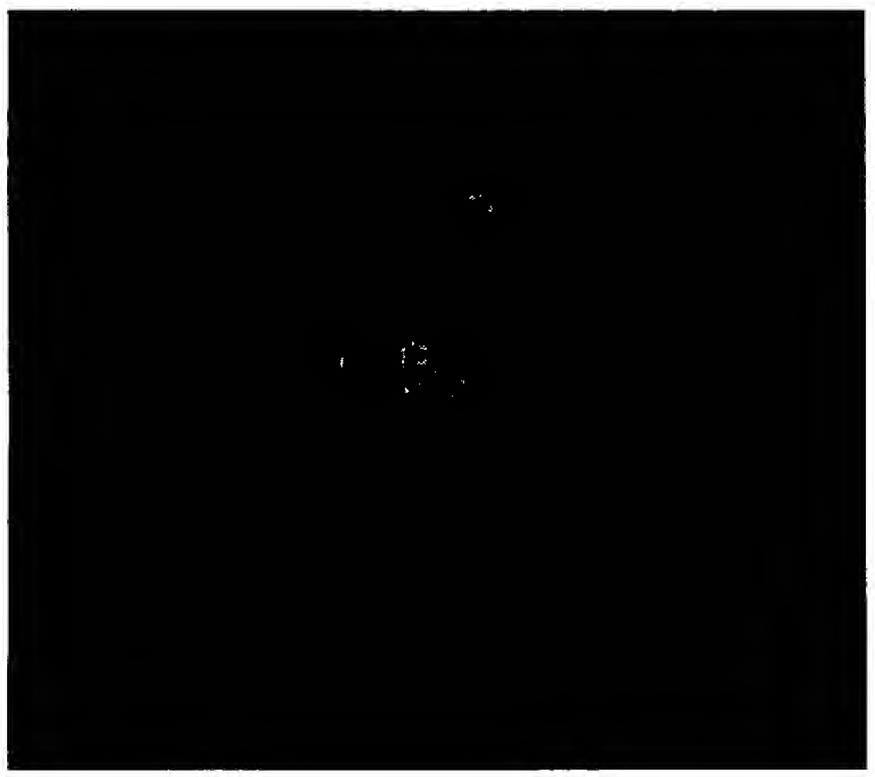
A. Flow Cytometry

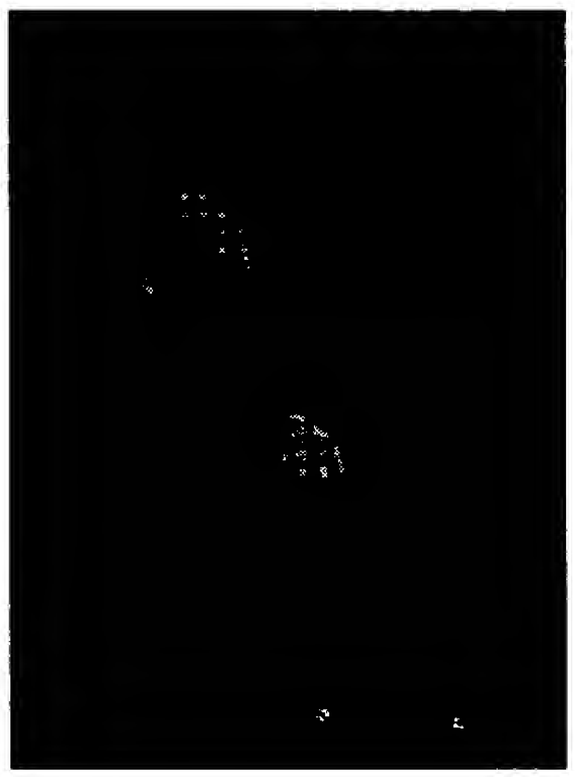


B. Fluorescent Microscopy

98P4B6.GFP.pcDNA3.1 transfected cells







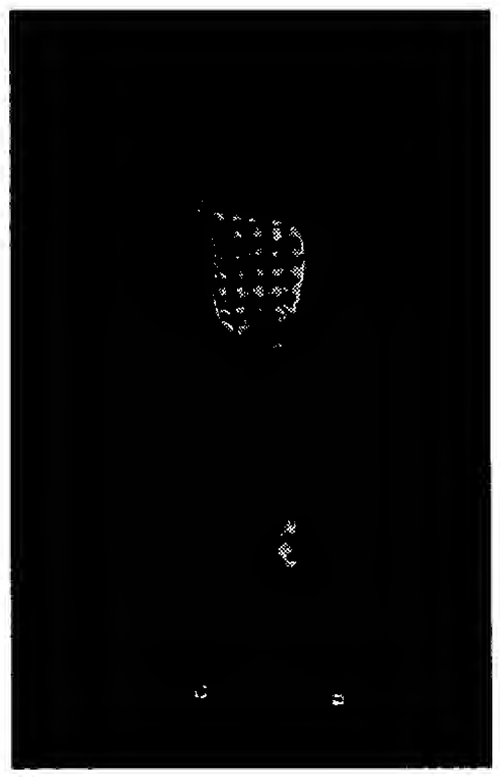
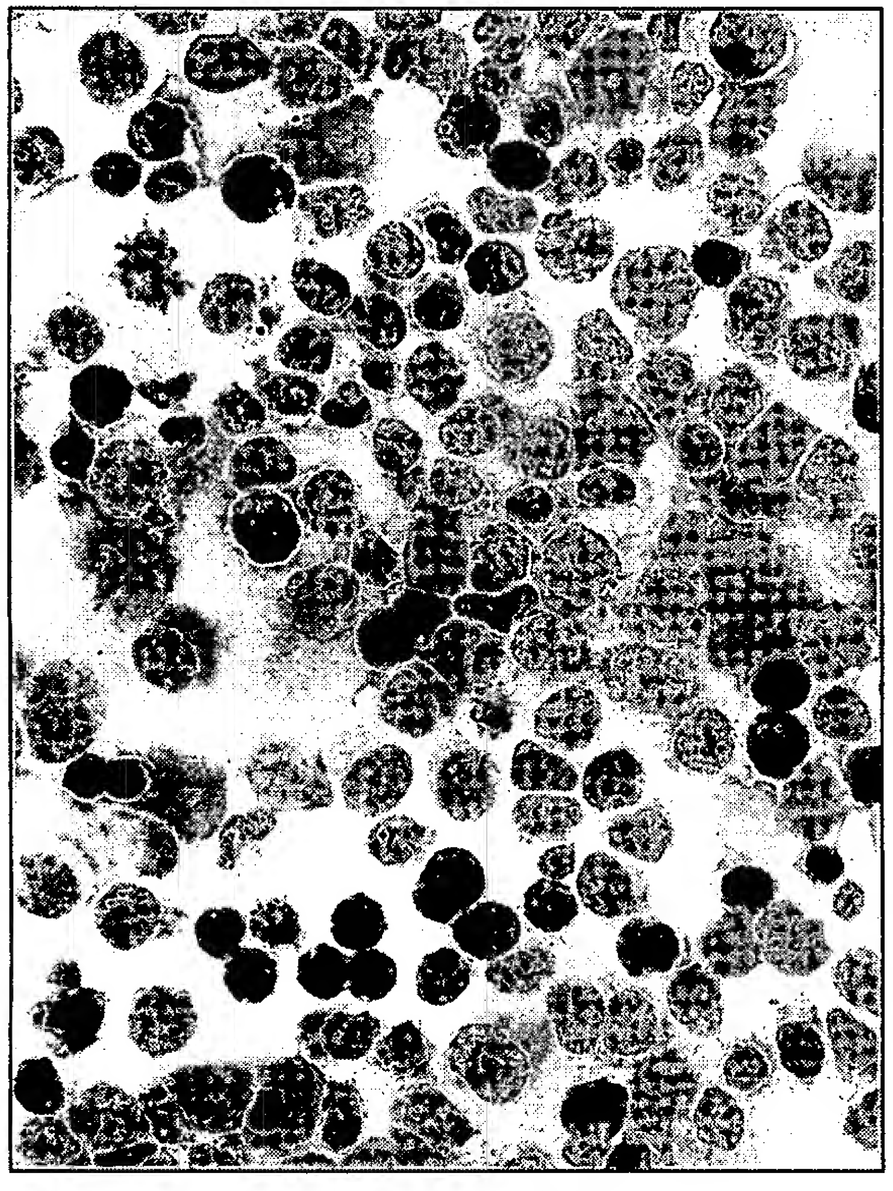


Exhibit 7: Panel A

A1.
STEAP2-293T cells



A2.
Vector-293T cells

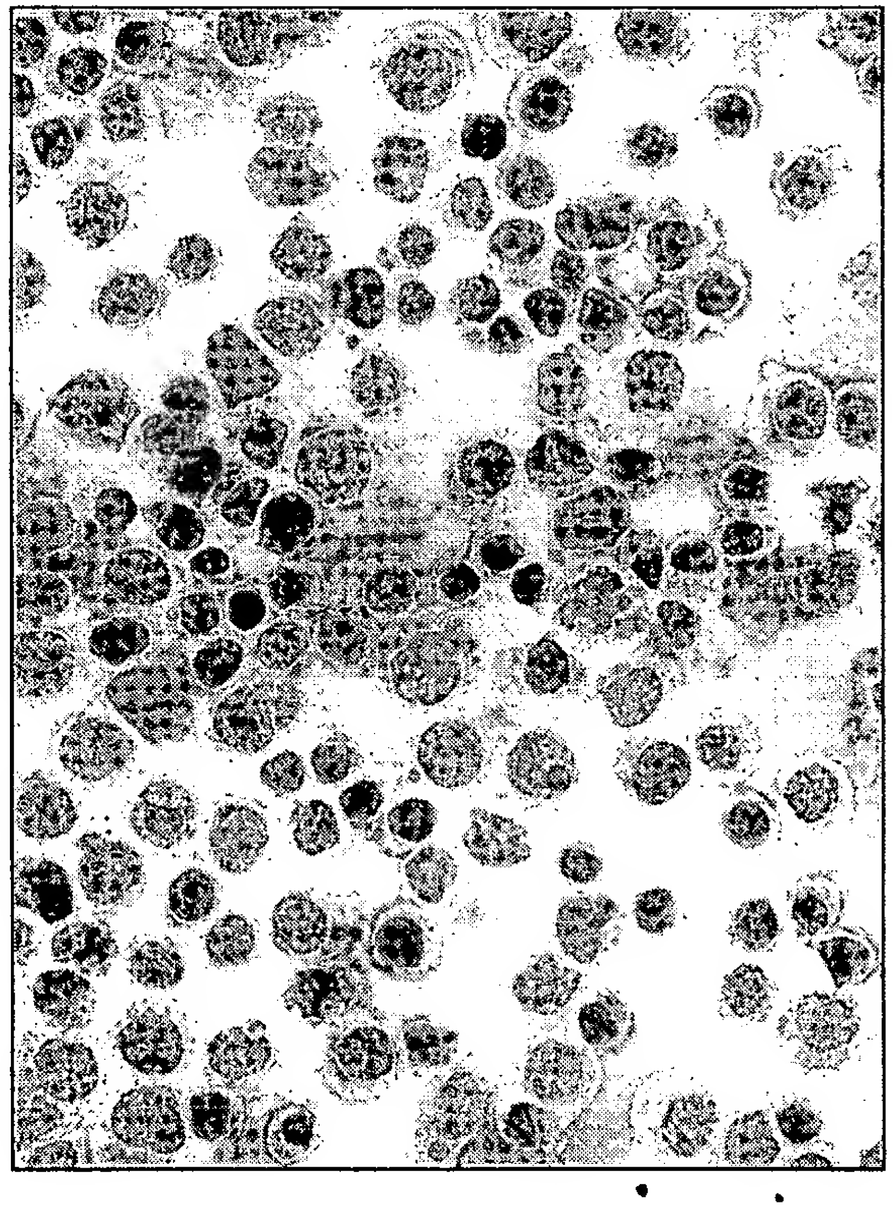
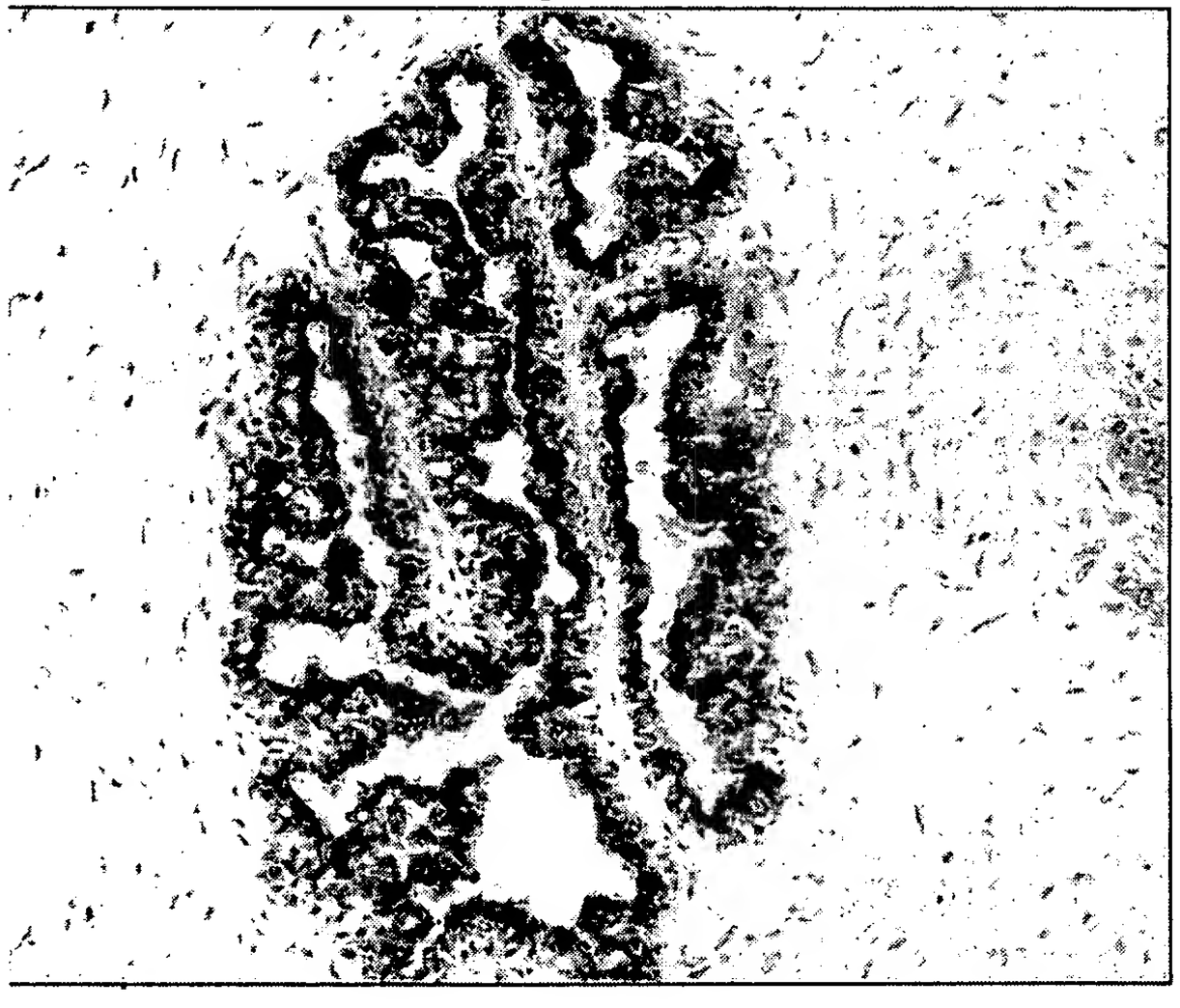


Exhibit 7: Panel B

B2.

)state cancer – gleason score 4



Lung cancer – adenocarcinoma